Please enter, /Jacob Cheu/
Oct 3, 2011

AMENDMENTS TO THE SPECIFICATION

In the Specification:

Please replace the first paragraph on page one of the specification with the following substitute paragraph:

This application is a U.S. National Stage Application of PCT/US04/26231, filed August 12, 2004, which claims priority to U.S. provisional patent application 60/495,193, filed ~~8/14/2003~~ August 14, 2003. The contents of the prior ~~application~~ applications are hereby incorporated by reference in their ~~entirety~~ entireties.

Please replace the third paragraph on page six of the specification with the following substitute paragraph:

Alternatively, an alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman (Smith and Waterman, 1981, Advances in Applied Mathematics 2:482-489; database: European Bioinformatics Institute; Smith and Waterman, 1981, J. of Molec. Biol., 147:195197; Nicholas et al., 1998, "A Tutorial on Searching Sequence Databases and Sequence Scoring Methods" (~~www.psc.edu~~) and references cited therein[[.]]; W.R. Pearson, 1991, Genomics 11:635-650). This algorithm can be applied to amino acid sequences by using the scoring matrix developed by Dayhoff (Dayhoff: Atlas of Protein Sequences and Structure, M. O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA), and normalized by Gribskov (Gribskov 1986 Nucl. Acids Res. 14(6):6745-6763). The Smith-Waterman algorithm may be employed where default parameters are used for scoring (for example, gap open penalty of 12, gap extension penalty of two). From the data generated, the "Match" value reflects "sequence identity."

Please replace the third paragraph on page seven of the specification with the following substitute paragraph:

In other embodiments, moderately stringent hybridization conditions are used that are: pretreatment of filters containing nucleic acid for 6 h at 40° C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HC1 (pH7.5), 5mM EDTA, 0.1% PVP, 0.1% ~~Ficoll~~ FICOLL®, 1% BSA, and 500 µg/ml denatured salmon sperm DNA; hybridization for 18-20h at 40° C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH7.5), 5mM EDTA, 0.02% PVP, 0.02% ~~Ficoll~~ FICOLL®, 0.2% BSA, 100 µg/m1 salmon sperm DNA, and 10% (wt/vol) dextran sulfate; followed by washing twice for 1 hour at 55° C in a solution containing 2X SSC and 0.1% SDS.

Please replace the fourth paragraph on page fifteen of the specification with the following substitute paragraph:

Antibodies that specifically bind MELK polypeptides can be generated using known methods. Preferably the antibody is specific to a mammalian ortholog of MELK polypeptide, and more preferably, to human MELK. Antibodies may be polyclonal, monoclonal (mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, ~~F(ab').sub.2~~ $F(ab')_2$ fragments, fragments produced by a ~~FAb~~ Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. Epitopes of MELK which are particularly antigenic can be selected, for example, by routine screening of MELK polypeptides for antigenicity or by applying a theoretical method for selecting antigenic regions of a protein (Hopp and Wood (1981), Proc. ~~Nati~~ Natl. Acad. Sci. U.S.A. 78:3824-28; Hopp and Wood, (1983) Mol. Immunol. 20:483-89; Sutcliffe et al., (1983) Science 219:660-66) to the amino acid sequence of a MELK. Monoclonal antibodies with affinities of $10^8 M^{-1}$ preferably $10^9 M^{-1}$ to $10^{10} M^{-1}$, or stronger can be made by standard procedures as described (Harlow and Lane, *supra;* Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed) Academic Press, New York; and U.S. Pat. Nos. 4,381,292; 4,451,570; and 4,618,577). Antibodies may be generated against crude cell extracts of MELK or substantially purified fragments thereof. If MELK fragments are used, they preferably comprise at least 10, and more preferably, at least 20 contiguous amino acids of a

MELK protein. In a particular embodiment, MELK- specific antigens and/or immunogens are coupled to carrier proteins that stimulate the immune response. For example, the subject polypeptides are covalently coupled to the keyhole limpet hemocyanin (KLH) carrier, and the conjugate is emulsified in Freund's complete adjuvant, which enhances the immune response. An appropriate immune system such as a laboratory rabbit or mouse is immunized according to conventional protocols.

Please replace the second paragraph on page sixteen of the specification with the following substitute paragraph:

The presence of MELK-specific antibodies is assayed by an appropriate assay such as a solid phase enzyme-linked immunosorbant assay (ELISA®) using immobilized corresponding MELK polypeptides. Other assays, such as radioimmunoassays or fluorescent assays might also be used.

Please replace the fourth paragraph on page twenty-two of the specification with the following substitute paragraph:

Apoptosis assays. Apoptosis or programmed cell death is a suicide program that is activated within the cell, leading to fragmentation of DNA, shrinkage of the cytoplasm, membrane changes and cell death. Apoptosis is mediated by proteolytic enzymes of the caspase family. Many of the altering parameters of a cell are measurable during apoptosis. Assays for apoptosis may be performed by terminal deoxynucleotidyl transferase-mediated digoxigenin-11-dUTP nick end labeling (TUNEL) assay. The TUNEL assay is used to measure nuclear DNA fragmentation characteristic of apoptosis (Lazebnik et al., 1994, Nature 371, 346), by following the incorporation of fluorescein-dUTP (Yonehara el al., 1989, J. Exp. Med. 169, 1747). Apoptosis may further be assayed by acridine orange staining of tissue culture cells (Lucas, R., et al., 1998, Blood 15:4730-41). Other cell-based apoptosis assays include the caspase-3/7 assay and the cell death nucleosome ELISA® assay. The caspase 3/7 assay is based on the activation of the caspase cleavage activity as part of a cascade of events that occur during programmed cell death in many apoptotic pathways. In the caspase 3/7 assay (commercially available ~~Apo~~ APO-ONE™ Homogeneous Caspase-3/7 assay from Promega, cat# 67790), lysis buffer and caspase substrate are mixed and added to

cells. The caspase substrate becomes fluorescent when cleaved by active caspase 3/7. The nucleosome ELISA® assay is a general cell death assay known to those skilled in the art, and available commercially (Roche, Cat# 1774425). This assay is a quantitative sandwich-enzyme-immunoassay which uses monoclonal antibodies directed against DNA and histones respectively, thus specifically determining the amount of mono- and oligonucleosomes in the cytoplasmic fraction of cell lysates. Mono and oligonucleosomes are enriched in the cytoplasm during apoptosis due to the fact that DNA fragmentation occurs several hours before the plasma membrane breaks down, allowing for ~~accumalation~~ accumulation in the cytoplasm. Nucleosomes are not present in the cytoplasmic fraction of cells that are not undergoing apoptosis. The Phospho-histone H2B assay is another apoptosis assay, based on phosphorylation of histone H2B as a result of apoptosis. Fluorescent dyes that are associated with phosphohistone H2B may be used to measure the increase of phosphohistone H2B as a result of apoptosis. Apoptosis assays that simultaneously measure multiple parameters associated with apoptosis have also been developed. In such assays, various cellular parameters that can be associated with antibodies or fluorescent dyes, and that mark various stages of apoptosis are labeled, and the results are measured using instruments such as ~~Cellomics~~ ~~CELLOMICS~~™ ~~ArrayScan~~ ARRAYSCAN® HCS System. The measurable parameters and their markers include anti-active caspase-3 antibody which marks intermediate stage apoptosis, anti-PARP-p85 antibody (cleaved PARP) which marks late stage apoptosis, Hoechst labels which label the nucleus and are used to measure nuclear swelling as a measure of early apoptosis and nuclear condensation as a measure of late apoptosis, TOTO-3 fluorescent dye which labels DNA of dead cells with high cell membrane permeability.

Please replace the third paragraph on page twenty-four of the specification with the following substitute paragraph:

Cell proliferation is also assayed via phospho-histone H3 staining, which identifies a cell population undergoing mitosis by phosphorylation of histone H3. Phosphorylation of histone H3 at serine 10 is detected using an antibody ~~specfic~~ specific to the phosphorylated form of the serine 10 residue of histone H3. (Chadlee, D.N. 1995, J. Biol. Chem 270:20098-105). Cell ~~Proliferation~~ proliferation may also be examined

using [^{3}H]- thymidine incorporation (Chen, J., 1996, Oncogene 13:1395-403; Jeoung, J., 1995, J. Biol. Chem. 270:18367-73). This assay allows for quantitative characterization of S- phase DNA syntheses. In this assay, cells synthesizing DNA will incorporate [^{3}H]-thymidine into newly synthesized DNA. Incorporation can then be measured by standard techniques such as by counting of radioisotope in a scintillation counter (e.g., Beckman LS 3800 Liquid Scintillation Counter). Another proliferation assay uses the dye Alamar Blue (available from Biosource International), which fluoresces when reduced in living cells and provides an indirect measurement of cell number (Voytik-Harbin SL et al., 1998, In Vitro Cell Dev Biol Anim 34:239-46). Yet another proliferation assay, the MTS assay, is based on in vitro cytotoxicity assessment of industrial chemicals, and uses the soluble tetrazolium salt, MTS. MTS assays are commercially available, for example, the Promega ~~CellTiter~~ CELLTITER 96® ~~AQueous~~ Aqueous Non-Radioactive Cell Proliferation Assay (Cat.# G5421).

Please replace the second paragraph on page twenty-five of the specification with the following substitute paragraph:

Cell proliferation may also be assayed by measuring ATP levels as indicator of metabolically active cells. Such assays are commercially available, for example ~~Cell Titer-Glo~~ CELL TITER-GLO ™, which is a luminescent homogeneous assay available from Promega.

Please replace the fifth paragraph on page twenty-five of the specification with the following substitute paragraph:

Angiogenesis. Angiogenesis may be assayed using various human endothelial cell systems, such as umbilical vein, coronary artery, or dermal cells. Suitable assays include Alamar Blue based assays (available from Biosource International) to measure proliferation; migration assays using fluorescent molecules, such as the use of Becton Dickinson Falcon HTS FluoroBlock cell culture inserts to measure migration of cells through membranes in the presence or absence of angiogenesis enhancer or suppressors; and tubule formation assays based on the formation of tubular structures by endothelial cells on ~~Matrigel~~ MATRIGEL® (Becton Dickinson). Accordingly, an angiogenesis assay system may comprise a

cell that expresses a MELK, and that optionally has defective RAC function (e.g. RAC is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the angiogenesis assay system and changes in angiogenesis relative to controls where no test agent is added, identify candidate RAC modulating agents. In some embodiments of the invention, the angiogenesis assay may be used as a secondary assay to test a candidate RAC modulating agents that is initially identified using another assay system. An angiogenesis assay may also be used to test whether MELK function plays a direct role in cell proliferation. For example, an angiogenesis assay may be performed on cells that over- or under-express MELK relative to wild type cells. Differences in angiogenesis compared to wild type cells ~~suggests~~ suggest that the MELK plays a direct role in angiogenesis. U.S. Pat. Nos. 5,976,782, 6,225,118 and 6,444,434, among others, describe various angiogenesis assays.

Please replace the second paragraph on page twenty-six of the specification with the following substitute paragraph:

Hypoxic induction. The alpha subunit of the transcription factor, hypoxia inducible factor-1 (HIF-1), is upregulated in tumor cells following exposure to hypoxia in vitro. Under hypoxic conditions, HIF-1 stimulates the expression of genes known to be important in tumour cell survival, such as those encoding ~~glyolytic~~ glycolytic enzymes and VEGF. Induction of such genes by hypoxic conditions may be assayed by growing cells transfected with MELK in hypoxic conditions (such as with 0.1% O2, 5% CO2, and balance N2, generated in a ~~Napco~~ NAPCO® 7001 incubator (Precision Scientific)) and normoxic conditions, followed by assessment of gene activity or expression by ~~Taqman~~ TAQMAN®. For example, a hypoxic induction assay system may comprise a cell that expresses a MELK, and that optionally has defective RAC function (e.g. RAC is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the hypoxic induction assay system and changes in hypoxic response relative to controls where no test agent is added, identify candidate RAC modulating agents. In some embodiments of the invention, the hypoxic induction assay may be used as a secondary assay to test a candidate RAC modulating ~~agents~~ agent that is initially identified using another assay system. A hypoxic induction assay may also be used to test whether MELK function plays a direct role in the hypoxic

response. For example, a hypoxic induction assay may be performed on cells that over- or under-express MELK relative to wild type cells. Differences in hypoxic response compared to wild type cells ~~suggests~~ suggest that the MELK plays a direct role in hypoxic induction.

Please replace the fourth paragraph on page twenty-eight of the specification with the following substitute paragraph:

Tubulogenesis. Tubulogenesis assays monitor the ability of cultured cells, generally endothelial cells, to form tubular structures on a matrix substrate, which generally simulates the environment of the extracellular matrix. Exemplary substrates include ~~Matrigel~~ MATRIGEL™ (Becton Dickinson), an extract of basement membrane proteins containing laminin, collagen IV, and heparin sulfate proteoglycan, which is liquid at 4° C and forms a solid gel at 37° C. Other suitable matrices comprise extracellular components such as collagen, fibronectin, and/or fibrin. Cells are stimulated with a pro-angiogenic stimulant, and their ability to form tubules is detected by imaging. Tubules can generally be detected after an overnight incubation with stimuli, but longer or shorter time frames may also be used. Tube formation assays are well known in the art (e.g., Jones MK et al., 1999, Nature Medicine 5:1418-1423). These assays have traditionally involved stimulation with serum or with the growth factors FGP or VEGF. Serum represents an undefined source of growth factors. In a preferred embodiment, the assay is performed with cells cultured in serum free medium, in order to control which process or pathway a candidate agent modulates. Moreover, we have found that different target genes respond differently to stimulation with different pro-angiogenic agents, including inflammatory angiogenic factors such as TNF-~~alpa~~ alpha. Thus, in a further preferred embodiment, a tubulogenesis assay system comprises testing a MELK's response to a variety of factors, such as FGF, VEGF, phorbol myristate acetate (PMA), TNF-alpha, ephrin, etc.

Please replace the second paragraph on page twenty-nine of the specification with the following substitute paragraph:

For antibody modulators, appropriate primary assays test is a binding assay that tests the antibody's affinity to and specificity for the MELK protein. Methods for testing antibody affinity and specificity are well known in the art (Harlow and Lane, 1988, 1999,

supra). The enzyme-linked immunosorbant assay (ELISA®) is a preferred method for detecting MELK-specific antibodies; others include FACS assays, radioimmunoassays, and fluorescent assays.

Please replace the fourth paragraph on page twenty-nine of the specification with the following substitute paragraph:

For nucleic acid modulators, primary assays may test the ability of the nucleic acid modulator to inhibit or enhance MELK gene expression, preferably mRNA expression. In general, expression analysis comprises comparing MELK expression in like populations of cells (e.g., two pools of cells that endogenously or recombinantly express MELK) in the presence and absence of the nucleic acid modulator. Methods for analyzing mRNA and protein expression are well known in the art. For instance, Northern blotting, slot blotting, ribonuclease protection, quantitative RT-PCR (e.g., using the ~~TaqMan~~ TAQMAN®, PE ~~Applied Biosystems~~ APPLIED BIOSYSTEMS®), or microarray analysis may be used to confirm that MELK mRNA expression is reduced in cells treated with the nucleic acid modulator (e.g., Current Protocols in Molecular Biology (1994) Ausubel FM *et al., eds.,* John Wiley & Sons, Inc., chapter 4; Freeman WM *et al.,* Biotechniques (1999) 26:112-125; Kallionierni OP, Ann Med 2001, 33:142-147; Blohm DH and Guiseppi-Elie, A. Curr Opin Biotechnol 2001, 12:41-47). Protein expression may also be monitored. Proteins are most commonly detected with specific antibodies or antisera directed against either the MELK protein or specific peptides. A variety of means including Western blotting, ELISA®, or in situ detection, are available (Harlow E and Lane D, 1988 and 1999, *supra).*

Please replace the second paragraph on page thirty-one of the specification with the following substitute paragraph:

In a preferred embodiment, RAC pathway activity is assessed by monitoring neovascularization and angiogenesis. Animal models with defective and normal RAC are used to test the candidate modulator's ~~affect~~ effect on MELK in ~~Matrigel~~ MATRIGEL® assays. ~~Matrigel~~ MATRIGEL® is an extract of basement membrane proteins, and is composed primarily of laminin, collagen IV, and heparin sulfate

proteoglycan. It is provided as a sterile liquid at 4°C, but rapidly forms a solid gel at 37°C. Liquid ~~Matrigel~~ MATRIGEL® is mixed with various angiogenic agents, such as bFGF and VEGF, or with human tumor cells which over-express the MELK. The mixture is then injected subcutaneously (SC) into female athymic nude mice (Taconic, Germantown, NY) to support an intense vascular response. Mice with ~~Matrigel~~ MATRIGEL® pellets may be dosed via oral (PO), intraperitoneal (IF), or intravenous (IV) routes with the candidate modulator. Mice are euthanized 5-12 days post-injection, and the ~~Matrigel~~ MATRIGEL® pellet is harvested for hemoglobin analysis (Sigma plasma hemoglobin kit). Hemoglobin content of the gel is found to correlate the degree of neovascularization in the gel.

Please replace the third paragraph on page thirty-three of the specification with the following substitute paragraph:

Various expression analysis methods can be used to diagnose whether MELK expression occurs in a particular sample, including Northern blotting, slot blotting, ribonuclease protection, quantitative RT-PCR, and microarray analysis. (e.g., Current Protocols in Molecular Biology (1994) Ausubel FM *et al., eds.,* John Wiley & Sons, Inc., chapter 4; Freeman WM *et al.,* Biotechniques (1999) 26:112-125; Kallioniemi OP, Ann Med 2001, 33:142-147; Blohm and Guiseppi-Elie, Curr Opin Biotechnol 2001, 12:41-47). Tissues having a disease or disorder implicating defective RAC signaling that express a MELK, are identified as amenable to treatment with a MELK modulating agent. In a preferred application, the RAC defective tissue overexpresses a MELK relative to normal tissue. For example, a Northern blot analysis of mRNA from tumor and normal cell lines, or from tumor and matching normal tissue samples from the same patient, using full or partial MELK cDNA sequences as probes, can determine whether particular tumors express or overexpress MELK. Alternatively, the ~~TaqMan~~ TAQMAN® is used for quantitative RT-PCR analysis of MELK expression in cell lines, normal tissues and tumor samples (PE ~~Applied Biosystems~~ APPLIED BIOSYSTEMS®).

Please replace the third paragraph on page thirty-seven of the specification with the following substitute paragraph:

All cell lines used in the following experiments are NCI (National Cancer Institute) lines, and are available from ATCC[SM] (American Type Culture Collection, Manassas, VA 20110-2209). Normal and tumor tissues were obtained from Impath, UC Davis, ~~Clontech~~ CLONTECH[TM], ~~Stratagene~~ STRATAGENE®, Ardais, Genome Collaborative, and ~~Ambion~~ AMBION®.

Please replace the fourth paragraph on page thirty-seven of the specification with the following substitute paragraph:

~~TaqMan~~ TAQMAN® analysis was used to assess expression levels of the disclosed genes in various samples.

Please replace the fifth paragraph on page thirty-seven of the specification with the following substitute paragraph:

RNA was extracted from each tissue sample using ~~Qiagen~~ QIAGEN[TM] (Valencia, CA) ~~RNeasy~~ RNEASY® kits, following manufacturer's protocols, to a final concentration of 50ng/µl. Single stranded cDNA was then synthesized by reverse transcribing the RNA samples using random hexamers and 500ng of total RNA per reaction, following protocol 4304965 of ~~Applied Biosystems~~ APPLIED BIOSYSTEMS® (Foster City, CA).

Please replace the first paragraph on page thirty-eight of the specification with the following substitute paragraph:

Primers for expression analysis using ~~TaqMan~~ TAQMAN® assay (~~Applied Biosystems~~ APPLIED BIOSYSTEMS®, Foster City, CA) are prepared according to the ~~TaqMan~~ TAQMAN® protocols, and the following criteria: a) primer pairs were designed to span introns to eliminate genomic contamination, and b) each primer pair produced only one product. Expression analysis was performed using 7900HT instrument.

Please replace the second paragraph on page thirty-eight of the specification with the following substitute paragraph:

~~TaqMan~~ TAQMAN® reactions are carried out following manufacturer's protocols, in 25 µl total volume for 96-well plates and 10 µl total volume for 384-well plates, using 300nM primer and 250 nM probe, and approximately 25ng of cDNA. The standard curve for result analysis is prepared using a universal pool of human cDNA samples, which is a mixture of cDNAs from a wide variety of tissues so that the chance that a target will be present in appreciable amounts is good. The raw data are normalized using 18S rRNA (universally expressed in all tissues and cells).